IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Geoffrey N. Roth and Jonathan N. Roth

Serial No:

Filed: January 7, 2002

Art Unit: 1631

For: TEST MEDIA AND QUANTITATIVE OR QUALITATIVE METHOD FOR IDENTIFICATION AND DIFFERENTIATION OF BIOLOGICAL MATERIALS IN A TEST SAMPLE

Commissioner for Patents and Trademarks
Washington DC 20231

Dear Sir or Madam:

DECLARATION OF JONATHAN N. ROTH UNDER RULE 132

I, Jonathan N. Roth, declare that I am one of the inventors of the invention disclosed and claimed for the Continuation Application of U.S. Application Serial Number 09/357,606, and that I have a Ph.D. in Phytopathology and Biochemistry and extensive experience in testing and evaluation for microbes. Attached are true and accurate copies of pages from an e-mail that I sent to Dr. David Sartory regarding his presentation at the 1992 Water Quality Technology Conference and his reply thereto (Attachment 1). In his reply, Dr. Sartory affirmed that in his experience the vast majority of glucuronidase-negative, oxidase-positive yellow colony isolates on m-LGA would be species of *Aeromonas*. Dr. Sartory also states that in another unpublished study he performed, only 1 of 203 isolates of *Aeromonas* from drinking water was glucuronidase-positive and only 2.7% of *Aeromonas* isolates from river water were glucuronidase-positive, which is in accord with the data of Kaznowski et al.

I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and like are made punishable by fine or prison or both, under Section 1001 of Title 18 of the United States Code, and that such willful statement and the like may jeopardize the validity of this patent application and any patent that issues therefrom.

Jan. 7, 2002
Date

Jonathan N. Roth
Jonathan N. Roth

Dani I Tychonievich - Fw: Re: glucuronidase production by Aeromonas spp

From: jonathan n roth <jonnr@juno.com>
To: <dtychoni@bakerd.com>
Date: 1/7/2002 8:32 AM
Subject: Fw: Re: glucuronidase production by Aeromonas

-------- Forwarded message --------
From: David.Sartory@severntrent.co.uk
To: jonathan n roth <jonnr@juno.com>
Date: Mon, 7 Jan 2002 11:42:28 +0000
Subject: Re: glucuronidase production by Aeromonas spp
Message-ID: <80256B3A.003F59CB.00@severntrent.co.uk>

Dear Jonathan,
Thank you for your interesting query regarding Aeromonas and the development of
m-LGA medium. Firstly, your interpretation of the data in my papers is correct.
Aeromonas are generally glucuronidase-negative, and the data presented in the
two papers does not indicate otherwise. Unfortunately there was a page
restriction for the Letters in Applied Microbiology paper, so I had to
concentrate on the E. coli and coliform data. I took the opportunity of the
AWWA WQTC paper to add the additional information gathered in the study. The
information on Aeromonas, however, was only supplementary showing that some
strains could give false-positive E. coli colonies. It should not be taken as
giving an indication of the glucuronidase-positive rate for aeromonads as only
selected isolates were tested (i.e. those that could give false-positive counts
for E. coli) and the medium is not intended for the recovery of Aeromonas.

The occurrence of 'blue colonies' on m-LGA is a very uncommon event, but we
looked at all those we had in case they were galactosidase-negative E. coli.
Although 13 were identified as
galactosidase-negative/glucuronidase-positive
Aeromonas and a further 2 'green colonies' as
galactosidase-positive/glucuronidase-positive Aeromonas, these would represent
only a small percentage of the Aeromonas occurring in our waters.

We did not identify the 133 oxidase-positive yellow colonies for that study, so
cannot say how many were glucuronidase-negative Aeromonas (although in our
experience the vast majority of oxidase-positive yellow colony isolates on m-LGA
would be species of Aeromonas). However, during a separate (unpublished) study
we undertook specifically on Aeromonas in drinking water, out of 203 isolates
from treated water (principally A. hydrophila with a few A. caviae, A. veronii
biogroup sobria and A. schubertii), only one was glucuronidase-positive (a
strain of A. hydrophila, which was also galactosidase-positive). Of 112 isolates

from river water, only 3 (2.7 %, all A. hydrophila) were glucuronidase-positive.
This accords with the data of Kaznowski et al. (1989), cited in the AWWA paper,
who give glucuronidase-positive rates of 3.1 % for A. hydrophila and 4.1 % for
A. sobria.

I hope this helps clarify the interpretation of my data.

With best regards,

David.

David Sartory
Company Advisor (Microbiology)
Quality & Environmental Services
Severn Trent Water Ltd
Welshpool Road
Shelton
Shrewsbury
SY3 8BJ
Tel: +44 (0) 1743 265765
Fax: +44 (0) 1743 265043
Mobile +44 (0) 7880 788208
E-mail: david.sartory@severntrent.co.uk

To: David Sartory/AssetMgt/STW/STPLC@SevernTrent
cc:
Fax to:
Subject: glucuronidase production by Aeromonas spp

Dear Dr. Sartory:

I am working in the area of water and food microbiology and am particularly interested in bacterial enzyme physiology as it pertains to applications in potential identification of various bacteria. Right now I am trying to resolve the question of whether or not members of the genus Aeromonas produce the enzyme glucuronidase, or if this genus in general is negative for glucuronidase production. The general references which I have found all seem to indicate that a very high percentage of Aeromonads are glucuronidase negative, but that an occasional strain is found which may produce the enzyme. Your work with Linda Howard which was reported in the 1992 Proceedings of the Water Quality Technology Conference of the AWWA in Toronto, Ontario (and which I believe were published in Letters in Applied Micro 1992, vol 15. 273-276) have been quoted to me as proof that Aeromonas species are glucuronidase positive as a general rule. I do not find in my reading of your work that you intended to indicate that is the case. In fact, my interpretation of your work would indicate otherwise, that most Aeromonas are glucuronidase negative. Am I wrong in my interpretation?

Also, in the published paper, you do not mention the 22 "blue" colonies on m-LGA (paragraph 4, page 1664), of which 13 were IDed as Aeromonas. You only speak of 2 green colonies in the paper. Also, on the m-LGA, of the 133 yellow colonies (table 1) which were oxidase +ve, did you do any testing for the % which were Aeromonads?

I am hopeful that you are willing and able to help me solve this difference of opinion and interpretation of your work.

Thanks very much for your help in clarifying this matter.

Jonathan Roth, Ph.D.
Professor of Biology
Goshen College